

Review

Graphene as Cancer Theranostic Tool: Progress and Future Challenges

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Abstract

Nowadays cancer remains one of the main causes of death in the world. Current diagnostic techniques need to be improved to provide earlier diagnosis and treatment. Traditional therapy approaches to cancer are limited by lack of specificity and systemic toxicity. In this scenario nanomaterials could be good allies to give more specific cancer treatment effectively reducing undesired side effects and giving at the same time accurate diagnosis and successful therapy. In this context, thanks to its unique physical and chemical properties, graphene, graphene oxide (GO) and reduced graphene (rGO) have recently attracted tremendous interest in biomedicine including cancer therapy.

Herein we analyzed all studies presented in literature related to cancer fight using graphene and graphene-based conjugates. In this context, we aimed at the full picture of the state of the art providing new inputs for future strategies in the cancer theranostic by using of graphene.

We found an impressive increasing interest in the material for cancer therapy and/or diagnosis. The majority of the works (73%) have been carried out on drug and gene delivery applications, following by photothermal therapy (32%), imaging (31%) and photodynamic therapy (10%). A 27% of the studies focused on theranostic applications. Part of the works here discussed contribute to the growth of the theranostic field covering the use of imaging (i.e. ultrasonography, positron electron tomography, and fluorescent imaging) combined to one or more therapeutic modalities. We found that the use of graphene in cancer theranostics is still in an early but rapidly growing stage of investigation. Any technology based on nanomaterials can significantly enhance their possibility to become the real revolution in medicine if combines diagnosis and therapy at the same time. We performed a comprehensive summary of the latest progress of graphene cancer fight and highlighted the future challenges and the innovative possible theranostic applications.

Key words: graphene, nanomedicine, carbon materials, cancer, tumor, theranostics, therapy.

Introduction

Despite the everyday progresses of medicine solutions for human health, today cancer is still one of the biggest challenges for humanity. Thanks to the advancements in prevention and in treatment, the survival rate has been improved in the last few years. However, cancer remains one of the main causes of

death worldwide with 8,2 million of death occurred in 2012. It is estimated that by 2020, there will be between 15 and 17 million new cases of cancer every year, 60% of which will be in developing countries [1]. In economical developed countries the burden of cancer is a result of population aging and growth as

well as an increasing adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and “westernized” diets [2, 3]. Cancer, as definition, is the uncontrolled growth of cells that can occur in any type of tissue and, at the late stage, these cells lose their adhesion capacities and migrate to healthy tissues. Other than surgical treatment, the different options are all based on a mechanical or pharmacological killing action against cancer cells, possibly avoiding the side effect damages of healthy cells.

Nanotechnology is one of the best promises to attack cancer cells more specifically, effectively and to reduce undesired side effects. In other terms, nanotechnology can be used to transport drugs to a specific site using specific keys such as antibodies. Moreover, in the context of developing innovative theranostics, nanomaterials could be used for imaging as a diagnostic tool and, at the same time, to stimulate and control the release of drugs in the cancer site.

In the recent years numerous nanomaterials have been explored for potential theranostic applications for cancer therapy thanks to their properties [4].

Compared to traditional molecular contrast agents or drugs, nanomaterials can be engineered to improve and integrate multiple functions in a single system also to give the control of drugs release, being of hope for the building of a next generation of anti-cancer tools [5].

The relatively new nanomaterial, graphene, has attracted tremendous interest in the scientific community and in the public [6-9] being explored for many potential applications due to its unique physico-chemical characteristics including electronic, optical, thermal and mechanical properties [10-12]. The precise structure of graphene has been the subject of debate over the years since it varies greatly with the preparation methods and extent of oxidation [13, 14]. Nevertheless, graphene can be rich in functional groups such as carboxylic and hydroxyl groups which facilitate its surface modifications. Very recently, graphene and graphene oxide (GO) have been investigated in a growing number of medical applications, such as drug delivery, diagnostics, tissue engineering and gene transfection all with the final aim to use it as a theranostic materials [15-18]. However, one of the main concerns of using graphene in nanomedicine is its biocompatibility. Similarly to many other nanomaterials, it is necessary to carefully address its bio-

degradability in aqueous solutions. In addition, the dimensions of the flakes of graphene could be responsible of different impacts on cell viability [19]. On the other hand, specific toxic effects of graphene on cancer cells could represent a positive point. Indeed, many reports have shown that this function of graphene could be useful in possible future therapeutic applications [20, 21], for example as an inhibitor of cancer cell metastasis [22]. Furthermore, different anticancer biomolecules such as siRNA, DNA and other drugs can be loaded onto the graphene surface for gene silencing and transfection, drug delivery and many other cancer therapy applications [23].

In this review we analyzed all studies presented in literature aiming to fight cancer using graphene and graphene-based conjugates. We found that the graphene strategies in fighting cancer can be summarized in 4 main groups: i) drug delivery, ii) photothermal therapy (PTT), iii) photodynamic therapy, (PDT) and iv) imaging. Furthermore, we evidenced the works where authors used diagnostic and different therapy strategies such as drug delivery into one system promoting the use of graphene as a theranostic tool. We also carefully evaluated the use and the impact of graphene by tumor type. Our purpose was to broaden the knowledge of graphene as useful technology for the future of clinical cancer treatment and diagnosis. In this work we point out what are the lacking areas of graphene investigation from an oncology point of view, underling what can be the most promising approaches for the use of graphene-based tools in the challenging field of cancer.

Studies selection criteria and overview

To achieve our aim, we performed a “PubMed search” using the following keywords: graphene, graphene oxide, cancer therapy, drug delivery and cancer, immunotherapy, imaging and cancer, cancer diagnosis. The keyword exploration was done in several different combinations. High impact review articles also served as additional tool. The list of reported studies includes all the retrieved publications from 2008 to November 2014. In **table 1** we report a characterization of all the studies based on: type of application, type of cancer, species, model, type of graphene in terms of functionalization, year of publication and reference.

Table 1. Functionalized graphene.

Type of applications	Type of cancer	Tumor-cell type	Model	Drug/Imaging molecules used	Graphene	Year	Reference
Drug Delivery and Imaging	Burkitt's Lymphoma	Human	<i>In Vitro</i>	Doxorubicin, Rituxan Fluorescence Imaging	nGO-PEG	2008	Sun X. et al (Nano Res.)
Imaging and Photothermal Therapy	Breast Cancer	Mouse	<i>In Vivo</i>	Fluorescence Imaging	nGS-PEG	2010	Yang K. et al (Nanoletters)

Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Doxorubicin and Camptothecin	nGO-Acid Folic	2010	Zhang L. et al (Small)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Camptothecin	GO-PVA	2011	Sahoo N.G. et al (Chem. Comm.)
Drug Delivery	Breast and Colon Cancer	Human	<i>In Vitro</i>	Maltodextrin (MD) and EA (ellagic acid)	GO-Pluronic F38, GO-Tween 80, GO-MD and EA	2011	Kakran M. et al (Curr. Med. Chem.)
Gene Delivery and Imaging	Cervical and Prostate Cancer	Human	<i>In Vitro</i>	pDNA (pCMV-Luc), Fluorescence Imaging	GO-BPEI	2011	Kim H. et al (Bioconjug Chem.)
Drug Delivery and Photothermal Therapy	Breast Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	nGO-PEG	2011	Zhang W. et al (Biomaterials)
Drug Delivery and Photodynamic Therapy	Cervical Cancer	Human	<i>In Vitro</i>	Chlorin e6	GO-PEG	2011	Tian B. et al (ACS Nano)
Photodynamic Therapy	Gastric Cancer	Human	<i>In Vitro</i>		GO-Folic Acid (FA)	2011	Huang P. et al (Theranostics)
Photothermal Therapy	Brain Cancer	Human	<i>In Vitro</i>		nano-rGO-PEG and RGD	2011	Robinson J.T. et al (J. Am. Chem. Soc.)
Imaging and Photothermal Therapy	Breast Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Fluorescence Imaging	rGO-QD (semiconductor quantum dots)	2012	Hu S.H. et al (Adv. Mater.)
Imaging and Photothermal Therapy	Breast Cancer	Mouse	<i>In Vivo</i>	Fluorescence, Photoacoustic, and MR imaging	rGO and rGO-iron chloride hexahydrate	2012	Yang K. et al (Adv. Mater.)
Drug Delivery	Brain Cancer	Human	<i>In Vitro</i>	1,3-bis(2-chloroethyl)-1-nitroso urea (BCNU)	GO-PAA	2012	Lu Y.J. et al (Int. J. Nanomedicine)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Doxorubicin	GN-PF127 (Pluronic F127)	2012	Hu H. et al (J. Biomed. Mater. Res. A)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Adriamycin	GO	2012	Wu J. et al (Nanotechnology)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	β -Lapachone	rGO-Fe ₃ O ₄	2012	Zheng X.T. et al (Mol. Pharm.)
Drug Delivery	Cervical Cancer	Mouse	<i>In Vitro</i>	Doxorubicin	NGs	2012	Yang Y. et al (Chemistry)
Drug Delivery	Cervical, Breast and Lung Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Tamoxifen Citrate	NGs	2012	Misra S.K. et al (Small)
Drug Delivery	Liver Carcinoma	Human	<i>In Vitro</i>	Elsinochrome A and Doxorubicin	rGO	2012	Wei G. et al (Chemistry)
Drug Delivery and Imaging	Breast Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	TRC105, Positron Emission Tomography	GO	2012	Hong H. et al (ACS Nano)
Imaging	Breast Cancer	Human	<i>In Vitro</i>	Fluorescence Imaging	GO-Fe ₃ O ₄ (Fe)-PAMAM-G4-NH ₂ -Cy5	2012	Wate P.S. et al (Nanotechnology)
Photothermal Therapy	Breast Cancer	Mouse	<i>In Vivo</i>		nGO-PEG	2012	Yang K. et al (Biomaterials)
Photothermal Therapy	Colon Cancer	Human	<i>In Vitro</i>		GT-rGO	2012	Abdolahad M et al. (Mater. Sci. Eng. C Mater. Biol. Appl.)
Imaging and Photothermal Therapy	Breast Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Photoacoustic imaging	rGO	2013	Sheng Z. et al (Biomaterials)
Imaging and Photothermal Therapy	Cervical Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	US imaging and X-ray CT imaging	GO-Au@PLA	2013	Jin Y. et al (Biomaterials)
Drug Delivery	Brain Cancer	Mouse	<i>In Vitro</i>	Camptothecin	GO-PDEA	2013	Kavitha T. et al (Phys. Chem. Chem. Phys.)
Drug Delivery	Brain Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	GO-PEG-Tf (Transferrin)	2013	Liu G. et al (ACS Appl. Mater. Interfaces)
Drug Delivery	Brain Cancer	Human	<i>In Vivo</i>	Epirubicin	NMGO-PEG	2013	Yang H.W. et al (Adv. Mater.)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Hematin-terminated dextran and Doxorubicin	GO	2013	Jin R. et al (ACS Appl. Mater. Interfaces)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Doxorubicin	GQDs	2013	Wang C. et al (Sci. Rep.)
Drug Delivery and Gene Delivery	Breast Cancer	Human	<i>In Vitro</i>	Adriamycin, siRNA (anti-miR-21)	GO-PEI-PSS	2013	Zhi F. et al (Plos One)
Drug Delivery	Cervical Cancer	Human	<i>In Vitro</i>	Doxorubicin	rGO-PEG-BPEI	2013	Kim H. et al (ACS Nano)
Drug Delivery	Cervical Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	GO-PEG-Tf (Transferrin)	2013	Liu C.W. et al (Biomacromolecules)
Drug Delivery	Cervical Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	rGO-CHA (cholesteryl hyaluronic acid)	2013	Miao W. et al (Biomaterials)
Drug Delivery	Liver Carcinoma	Human	<i>In Vitro</i>	5-fluorouracil	MGNs	2013	Fan X. et al (Nanoscale)
Drug Delivery	Lung Cancer	Human	<i>In Vitro</i>	Paclitaxel	GO	2013	Arya N. et al (Nanoscale)
Gene Delivery	Skin Cancer	Mouse	<i>In Vivo</i>	Stat3-specific siRNA	GO-PEI-PEG	2013	Yin D. et al (Nanotechnology)
Drug Delivery and Imaging	Breast Cancer	Human and Mouse	<i>In Vitro, In Vivo and Ex Vivo</i>	TRC105, Positron Emission Tomography	rGO	2013	Shi S. et al (Biomaterials)
Drug Delivery and Imaging	Cervical Cancer	Human	<i>In Vitro</i>	Doxorubicin, Fluorescence Imaging	GO-QDs	2013	Chen M.L. et al (Bioconjug. Chem.)
Drug Delivery and Imaging	Liver Carcinoma	Human	<i>In Vitro</i>	Doxorubicin, MR Imaging	GO-DTPA-Gd (diethylenetriaminepentaacetic acid-gadolinium)	2013	Zhang M. et al (ACS Appl. Mater. Interfaces)
Drug Delivery and Photodynamic Therapy	Skin cancer	Mouse	<i>In vitro and In Vivo</i>	Doxorubicin, Chlorin e6	GO	2013	Miao W. et al (Biomaterials)
Drug Delivery and Photothermal Therapy	Brain Cancer	Human	<i>In Vitro</i>	Doxorubicin	GSPI (silica-coated graphene nanosheet)	2013	Wang Y. et al (J. Am. Chem. Soc.)
Drug Delivery and Photothermal Therapy	Brain Cancer	Mouse	<i>In Vitro</i>	Epirubicin	GO-PEG-EGFR	2013	Yang H.W. et al (Biomaterials)
Photothermal, Photodynamic Therapy and Imaging	Breast Cancer	Human and Mouse	<i>In Vitro, In Vivo and Ex Vivo</i>	Upconversion luminescence imaging	GO-UCNPs--ZnPc	2013	Wang Y. et al (Biomaterials)
Drug Delivery and Photothermal Therapy	Cervical and Lung Cancer	Human	<i>In Vitro</i>	Doxorubicin	GO	2013	Qin X.C. et al (J Photochem Photobiol B.)

Drug Delivery and Photothermal Therapy	Prostate Cancer	Mouse	<i>In Vitro</i>	Doxorubicin, CGN (thermo-sensitive nanogel)	rGO	2013	Wang C. et al (Nanomedicine)
Imaging	Breast Cancer	Mouse	<i>In Vivo</i>	Computed Tomography	GO	2013	Cornelissen B. et al (Biomaterials)
Photothermal and Photodynamic Therapy	Cervical Cancer	Human	<i>In Vitro and In Vivo</i>		GO	2013	Sahu A. et al (Biomaterials)
Photothermal Therapy	Breast and Lung Cancer	Human	<i>In Vitro</i>		rGO-Cu ₂ O Nanocrystal	2013	Hou C. et al (Nanoscale)
Photothermal Therapy	Cervical Cancer	Human and Mouse	<i>In Vitro, In Vivo and Ex Vivo</i>		GO-IONPs-Au-PEG	2013	Shi X. et al (Biomaterials)
Drug Delivery and Imaging	Lung and Prostate Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin and GFP-plasmid, Fluorescence Imaging	CMG (Chitosan Magnetic-Graphene)	2013	Wang C et al. (J. Mater. Chem. B Mater. Biol. Med.)
Drug Delivery and Imaging	Liver Cancer	Human	<i>In Vitro</i>	Doxorubicin, MR imaging and Fluorescence imaging	GO-SiO ₂	2013	Gao Y et al (Colloids Surf. B Biointerfaces)
Gene Delivery and Photothermal Therapy	Cervical and Breast Cancer	Human	<i>In Vitro</i>	siRNA targeting Plk1 mRNA	GO-PEG-PEI	2013	Feng L et al (Small)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Anastrozole	GO-IOF/IOI/IO	2014	Chaudhari N.S. et al (Mater. Sci. Eng. C Mater. Biol. Appl.)
Drug Delivery	Breast Cancer	Human	<i>In Vitro</i>	Camptothecin	GO-cyclodextrin, hyaluronated adamantane	2014	Zhang Y.M. (Chem. Comm.)
Drug Delivery	Breast and Pancreatic Cancer	Human	<i>In Vitro</i>	Gambocic Acid	Gs/SWCNTs	2014	Saeed M.L. et al (J Appl Toxicol.)
Drug Delivery	Cervical Cancer	Human	<i>In Vitro</i>	Camptothecin	GO-PVCL	2014	Kavitha T. et al (Colloids Surf B Biointerfaces)
Drug Delivery	Cervical Cancer	Human	<i>In Vitro</i>	Curcumin, Paclitaxol, Camptothecin and Doxorubicin	NGs-nile red and C-folate.	2014	Maity A.R. et al (Nanoscale)
Drug Delivery	Cervical Cancer	Human	<i>In Vitro</i>	Doxorubicin	GO-PEI	2014	Chen H. et al (ACS Appl. Mater. Interface)
Gene Delivery	Colon Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	dsDNA	GO	2014	Joseph D. et al (ACS Appl. Mater. Interfaces)
Drug Delivery	Glioblastoma and Breast Cancer	Human and Mouse	<i>In Vitro</i>	Lucanthone	GO-PEG-DSPE	2014	Chaudhary S.M. et al (Nanomedicine)
Drug Delivery	Liver Carcinoma	Human	<i>In Vitro</i>	Camptothecin	GO	2014	Yang X. et al (Nanoscale)
Drug Delivery	Liver Carcinoma	Human and Mouse	<i>In Vitro and in Vivo</i>	Doxorubicin	GO-HA (hyaluronic acid)	2014	Song E. et al (ACS Appl. Mater. Interfaces)
Drug Delivery	Liver Carcinoma	Human	<i>In Vitro</i>	Doxorubicin	GO-PEG-alginate	2014	Zhao X. et al (Langmuir)
Drug Delivery	Lung Cancer	Human	<i>In Vitro</i>	Paclitaxel	GO-PEG	2014	Xu Z. et al (ACS Appl. Mater. Interfaces)
Drug Delivery and Imaging	Cervical Cancer	Human	<i>In Vitro</i>	Doxorubicin, Fluorescence Imaging	GO Capped Mesoporous Silica	2014	He D. et al (Langmuir)
Drug Delivery and Imaging	Colon Cancer	Human	<i>In Vitro and in Vivo</i>	Curcumin	NGs Quantum Dot	2014	Some S. et al (Sci. Rep.)
Drug Delivery and Imaging	Liver Carcinoma	Human	<i>In Vitro</i>	Curcumin, Optical Imaging	GO-RGD-Chitosan	2014	Wang C. et al (Colloids and Surf. B Biointerfaces)
Drug Delivery and Photodynamic Therapy	Lung Cancer	Human	<i>In Vitro</i>	Hypocrellin A and Camptothecin	rGO	2014	Zhou L. et al (J. Photochem. Photobiol. B)
Drug Delivery and Photothermal Therapy	Breast Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	GO-Au	2014	Shi J. et al (Biomaterials)
Drug Delivery and Photothermal Therapy	Cervical Cancer	Human and Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin	PEG-GO/Cus	2014	Bai J. et al (Biomaterials)
Drug Delivery and Photothermal Therapy	Cervical Cancer	Human	<i>In Vitro</i>	Doxorubicin	rGO Capped Mesoporous Silica	2014	Wan H. et al (Nanoscale)
Drug Delivery, Imaging and Photothermal Therapy	Breast Cancer	Human	<i>In Vitro</i>	Doxorubicin	NGsAu nanocrystal	2014	Bian X. et al (Sci. Rep.)
Imaging	Breast Cancer	Human	<i>In Vitro</i>	Colorimetric Assay	GO-PtNPs (porous platinum nanoparticles)	2014	Zhang L.N. et al (Anal. Chem.)
Photodynamic Therapy and Imaging	Cervical Cancer	Human	<i>In Vitro</i>	Fluorescence Imaging	NGs-QDs	2014	Ge J. et al (Nat Commun.)
Photothermal Therapy and Imaging	Pancreatic cancer	Human	<i>In Vitro and In Vivo</i>	MR imaging	GO-ION	2014	Wang S. et al (Biomaterials)
Photodynamic Therapy	Breast Cancer	Mouse	<i>In Vitro</i>		GO-PEG	2014	Rong P. et al (Theranostics)
Photothermal, Photodynamic Therapy and Imaging	Cervical Cancer	Human	<i>In Vitro</i>	Fluorescence and MR imaging	MFG (magnetic and fluorescent graphene)	2014	Gollavelli G. et al (Biomaterials)
Photothermal Therapy and Imaging	Breast Cancer	Human	<i>In Vitro</i>	Reman Imaging	GO and GOAuNS	2014	Nergiz S.Z. et al (ACS Appl. Mater. Interfaces)
Photothermal Therapy	Epidermoid Carcinoma	Human and Mouse	<i>In Vitro and In Vivo</i>		GO-PEG-AuNR	2014	Dembereldorj U. et al (Photochem Photobiol)
Drug Delivery and Imaging	Glioma	Human	<i>In Vitro</i>	Doxorubicin, MR imaging	MGSPI(Magnetic graphene mesoporous silica)	2014	Wang Y. et al (Small)
Drug Delivery	Colon Cancer	Mouse	<i>In Vitro and In Vivo</i>	Doxorubicin, Camptothecin, Oxaliplatin and Cisplatin	GO	2014	Chen GY et al (Biomaterials)
Drug Delivery and Photothermal Therapy	Breast Cancer	Human	<i>In Vitro</i>	Doxorubicin	GO-PEG-DA (2,3-dimethylmaleic anhydride)	2014	Feng L et al (Adv. Healthc. Mater.)
Photothermal Therapy	Gastric Cancer	Human	<i>In Vitro</i>		rGO, GO	2014	Li J.L. et al (J Biomed Mater Res A.)

The trend (Figure 1), from 2008 to 2014, shows an impressive increasing interest in graphene for cancer therapy; i.e. the number of publications in 2013 and 2014 triplicated from 2012. Focusing on the type of application, we found that the majority of the works (73%) have been carried out on drug delivery and gene delivery (Figure 2A). The potential to act as a delivery tool against tumor cells seems to be one of the most attractive areas for scientists. In particular, compared to carbon nanotubes [24-26], graphene has two exposed side surface and, thus, at least a double external surface area than nanotubes that improve the conjugation capacity [27]. The particular arrangement of carbon atoms favors the *non covalent* complexation of drugs onto its surface, making possible a better release of drugs to the targeted cells. This characteristic can be one of the reasons why graphene has raised great success in drug delivery applications for cancer therapy. Intriguingly, part of the studies used GO for drug delivery combined with other purposes, such as imaging, acquiring the ability to perform and follow the drug release.

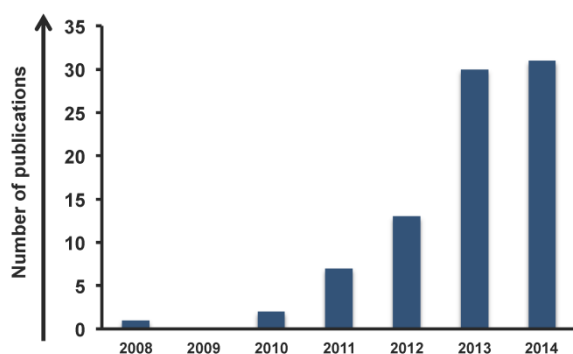


Figure 1. Percentage of publications of graphene in cancer fight (2008 to November 2014).

Photothermal therapy is the second biggest portion of works here analyzed with a portion of 32% (Figure 2A). In this context the material become attractive since it has a large surface area, is lightweight, exhibits high strength and electrical conductivity and is capable of generating plasmon, fluorescence, and nonlinear emission [5]. In particular, photothermal therapy uses the capacity of graphene to absorb light in the near-infrared region (NIR). Irradiation at 808 nm has been exploited, for example, in the ablation of many types of tumors both in vitro and in vivo in animal model [28].

Imaging application is in the third position in terms number of works related to cancer with 31% of the contributions (Figure 2A). Nanotechnology imaging is very fruitful field and in the last few years has attracted many researchers aiming at testing the characteristics of numerous nanomaterials, such as

carbon nanotubes [29] and quantum dots, as contrast agents [30] (Qdots) and graphene. Finally, a small part of the applications is occupied by photodynamic therapy (10%). The Venn diagram (Figure 2B), also shows 18 studies that used graphene for combining imaging and other cancer therapy, which further confirms and emphasizes the interest on this nanomaterial for cancer diagnosis and therapy at the same time. The works on graphene as theranostic tool cover the 27%.

Indeed, part of the works herein cited cover the use of imaging (ultrasonography, positron electron tomography (PET), fluorescent imaging) combined to one or more therapeutic action at the same time as showed in the Venn diagram (Figure 2B).

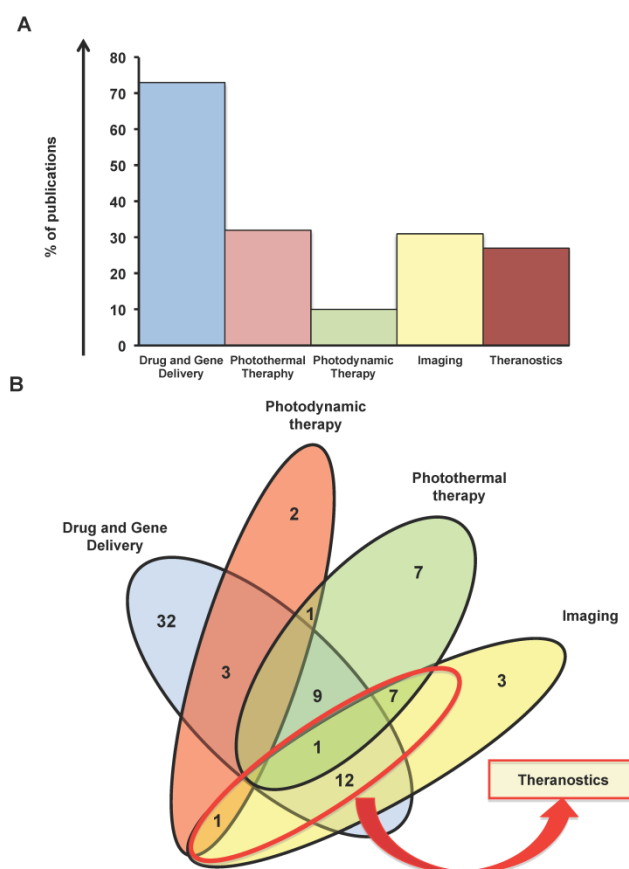


Figure 2. Status of Graphene publications in the last 7 years for cancer fight. A) Percentage of manuscripts based on the applications against cancer. B) Venn diagram based on the main applications (Drug Delivery, Photothermal therapy, Photodynamic therapy, Imaging). In the red round the theranostic studies.

We then decided to focus on the different types of cancer taken into consideration (Figure 3). Breast cancer is the most studied with a 35% of publications. Breast cancer is the most frequently diagnosed cancer in the world and the leading cause of cancer death in women, accounting for 25% (1.63 millions) of the total new cancer cases and 6,4% (0.522 millions) of the total

cancer deaths in 2012 [31]. About half of the breast cancer cases and 60% of the deaths are estimated to occur in Asian countries such as Iran, India and Qatar [2]. The second biggest portion is occupied by cervical cancer with a 23% of the total cases. Liver cancer is studied by 9% of the studies and the other cancers such as lymphoma, glioblastoma, glioma lung cancer, colon cancer, prostate cancer, brain cancer, pancreatic cancer and skin cancer takes the remaining part of the pie, with a range from 1% to 8% (**Figure 3**).

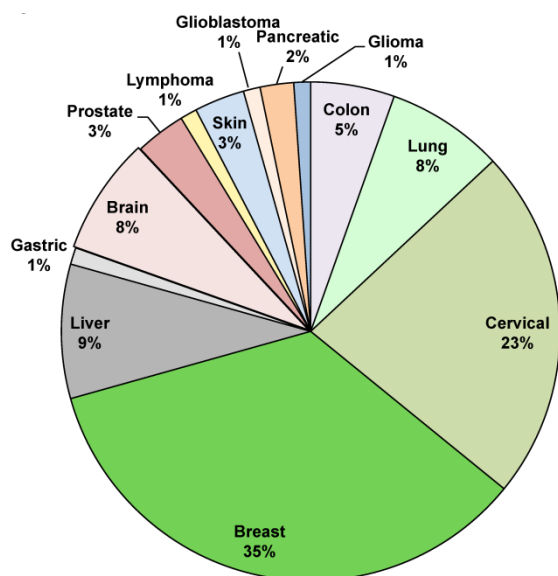


Figure 3. Overview on different type of cancer treated with graphene. Manuscripts percentages per type of studied cancer.

Drug and Gene Delivery

In recent years graphene and the other members of the family including GO and reduced GO (rGO) have been investigated for biological and biomedical applications thanks to their possible biocompatibility [32]. Moreover, the extremely large surface area of the material, with every atom exposed on its surface allow ultra-high drug and gene loading efficiency [27]. All these properties make graphene an optimal candidate as drug carrier and gene delivery system as reported in **table 1** [15, 32-92].

The good drug loading ability of graphene encouraged many researchers to explore it in many different types of cancer. According to the general trend, the most studied tumor, with 31% of the total works, is breast cancer (**Figure 5A**) [15, 32, 34, 36, 39-41, 43, 45, 49-51, 58, 65, 66, 72, 81, 86, 89, 92], followed by cervical cancer [35, 37, 42, 43, 52-54, 59, 63, 67, 68, 77, 78, 82, 89] and liver cancer [44, 55, 72-75, 79, 85, 88] with 23% and 10% respectively.

Other cancer types such as Burkitt's lymphoma [33], colon cancer [34, 70, 71, 78, 91], prostate cancer

[35, 64, 87], lung cancer [43, 56, 63, 76, 80, 87], skin cancer [57, 60], brain cancer [20, 38, 46-48, 61, 62], glioma [90] and glioblastoma [72] were studied under different drug treatment conjugated with graphene or graphene oxide.

Drug Delivery

As previously mentioned, drug delivery is the first application of graphene in terms of number of studies. We found 61 works that used graphene for drug and gene delivery alone or combined with other types of modalities to treat cancer (**Figure 2B**), such as PTT (10 works), PDT (3 works), and in imaging (13 works). A big challenge in this perspective is to perform a good drug functionalization. Indeed, different approaches have been applied to load drug molecules onto graphene by different binding strategies. Many studies used polyethylene glycol (PEG) to increase the biocompatibility and physiological stability of graphene or graphene oxide and subsequently load anticancer drugs via *non* covalent interaction [32]. Liu et al. [47] functionalized the surface of GO-PEG with different ligands such as transferrin and doxorubicin (DOX) to target brain tumors. The conjugated nanosystems with transferrin and doxorubicin displayed a greater intracellular delivery efficiency and stronger cytotoxicity against glioma.

Regarding GO, it is certainly more investigated compared to pristine graphene for drug delivery purposes. Zhang et al. [15] proposed a very innovative approach to exploit functionalized GO in biomedical research. The authors used first sulfonic acid groups functionalization (to make GO stable in physiological solutions) followed by covalent binding of folic acid targeting specifically MCF-7 cells, a human breast cancer cell line expressing folic acid receptors. They demonstrated that this system loaded with two anticancer drugs (DOX and camptothecin) showed specific targeting MCF-7 cells and a remarkably high cytotoxicity compared to the material only loaded with one of the two drugs.

DOX is a widely used chemotherapy agent and it can be loaded onto graphene via simple π - π stacking with high efficiency, resulting very promising to target the cancer. Thanks to this behavior, 31 studies were performed aiming at the delivery of DOX by graphene (**Figure 4**) [33, 36, 39, 42, 47, 49, 50, 53, 54, 61, 63, 64, 68, 69, 74, 75, 77, 81-85, 87, 88, 90-92]. One of these studies used GO as a potential alternative to cross blood brain barrier in order to destroy cancer cells by the action of DOX [47]. Another approach used by Zhang et al. [36] evidenced the possibility of GO-PEG-DOX conjugate to combine the local specific chemotherapy with external near-infrared (NIR) photothermal therapy, which significantly improved

the therapeutic efficacy of the cancer treatment. Moreover, GO was used to load two or more drugs onto its surface at the same time [15, 33, 44, 68].

We found camptotecin (CPT) as the second most conjugated drug to graphene (**Figure 4**). Sahoo *et al.*, for example, described GO-poly(vinyl alcohol) (PVA) as drug carrier for CPT via non covalent interactions [32]. GO-PVA-CPT exhibited higher cytotoxic activity versus cancer cells compared to CPT alone. A lot of drugs were used in multiple conjugations with graphene such as camptothecin [32, 66], 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) [38], a commercial chemotherapeutic drug for treating malignant brain tumors, tamoxifen citrate [43], elsinochrome A [44], adriamycin [40], β -lapachone [41], luncanthone [72], paclitaxel [56, 76], anastrozole [65], 5-fluorouracil [55], epirubicin [48, 62], curcumin [68], gambogic acid [86] ellagic acid [34], oxaliplatin (OXA) [91], cisplatin [91] and thermosensitive nanogel [64]. All these studies showed a great improvement in therapeutic efficacy when the drugs were loaded onto graphene. These promising data underline also the abilities of graphene as chemosensitizer. In this regard, Chen *et al.* tested GO with different drugs (DOX, CPT, OXA and cisplatin (CDDP)) for the treatment of colon cancer cells (CT26 cells). They have shown that GO tested together with CDDP dramatically decreased the cell viability compared to the CDDP alone on resistant cells. The authors attributed this behavior to the capacity of GO to induce moderate levels of autophagic flux and also to potentiate nuclear import of the autophagy marker LC3 and CDDP [70, 91].

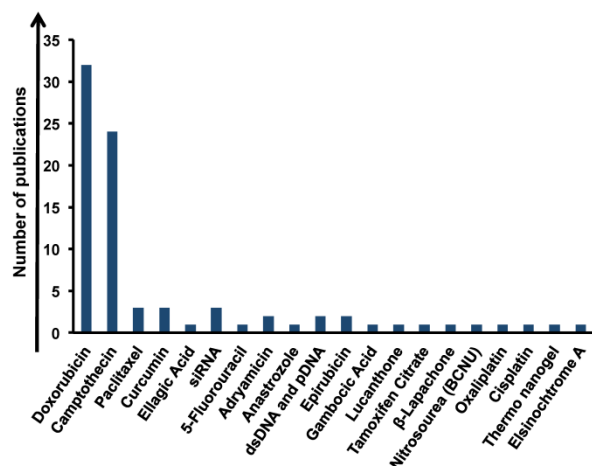


Figure 4. Analysis of the amount of publications of graphene in drug delivery applications based on type of loaded drugs.

Gene delivery

Graphene-based materials have been also widely used for gene therapy as smart gene (siRNA, dsDNA and antisense oligonucleotides) carriers, for their po-

tential in the treatment of gene related diseases including cancer [35, 51, 57, 71, 89]. Zhi *et al.*, for example, successfully used GO for co-delivery of drug (adriamycin) and siRNA against miRNA-21 (anti-miR-21) that is responsible of multidrug resistance in breast cancer cells. They found that the treatment with GO as a carrier of chemotherapeutic drugs and siRNA is favorable for the treatment of drug resistant cancers restoring the chemosensitivity of anticancer drugs [51]. Another remarkable study performed by Yin *et al.* focused on melanoma [57], an aggressive disease characterized by a complex etiology where immunotherapy and targeted therapy seems to be promising ways to fight it [93, 94]. In this context, the authors evidenced the use of GO as a carrier of plasmid-based Stat3 siRNA. Their results indicated significant regression in tumor growth and tumor weight after treatment without any collateral toxicity *in vivo* mouse model [57].

Photothermal Therapy

We found that the second most studied application is the photothermal therapy (PTT) (**Figure 2A**). Recent publications have shown the interesting potential of GO for PTT applications (see **Table 1**) [28, 36, 61-64, 81-84, 95-109]. PTT has been reported either alone [95, 98, 99, 105, 108-110] or in combination with drugs [36, 61, 62, 64, 82, 104, 111] or with PDT [102, 103, 106] or in both (**Figure 2B**). Photosensitizing agents are employed in PTT to generate heat from light absorption, leading to photoablation of cancer cells and subsequent cell death. To avoid nonspecific heating of healthy cells, photosensitizers must show absorption in the near-infrared region [112] and selective uptake in cancerous cells over normal cells. Deep penetration and negligible nonspecific photothermal heating in the NIR window are due to the transparency and low absorption of light by tissues in this optical window. Nowadays, a lot of nanomaterials are under investigation for their high optical absorbance in NIR for PTT including gold nanoshells [113], gold nanorods [114], gold pyramids [115], single-walled carbon nanotubes (SWCNTs), and multi-walled carbon nanotubes (MWCNTs) [116]. Robinson *et al.* [95] used rGO non covalently PEGylated and conjugated to a peptide for targeting and selective photoablation of cancer cells at a low doses. Abdolabad *et al.* [99] used rGO linked to the aromatic rings of green tea. Green tea is well known for its possible anticancer activity; indeed its polyphenol groups could be bound to cancer cell surface receptors. One of the important agents for this binding process is epigallocatechin gallate, the main polyphenol in green tea, which binds to the cancer cell surfaces. Thanks to the properties of green tea, the authors used low

concentration of reduced GO-green tea and had applied laser power in the PTT of colon cancer cells to obtain also high ablation efficiency [99]. It is also interesting to note that 7 studies [28, 84, 97, 100, 101, 107, 117] (**Figure 2B**) evidenced the use of graphene at the same time for imaging and PTT. Bian et al. [84] used graphene Au nanocrystals for combining PTT with imaging and DOX delivery against breast cancer cells. They performed a controlled release of DOX molecules from graphene nanocrystals through NIR heating, this system significantly reduced the possibility of side effects compared to general chemotherapy.

As for drug delivery applications, the most studied tumor for PTT graphene-based treatment is breast cancer with the 42% of the works [28, 36, 81, 96, 98, 100, 102, 104], followed by cervical cancer (26%) (**Figure 5B**) [37, 63, 82, 101, 103, 105, 106]. The other cancers treated with graphene in PTT modality comprise brain cancer (10%) [61, 62, 95] and lung cancer (7%) [63, 104]. The remaining 15% of studies [64, 99, 108, 109] focus on prostate, pancreatic skin, colon and gastric cancer (**Figure 5B**).

We found a growing interest in graphene breast cancer applications. Zhue et al. [118] i.e. discovered

that GO was able to selectively down-regulate PGC-1 α in breast cancer cells with a consequent inhibition of ATP production. Furthermore, GO was able to impair the assembly of the F-actin cytoskeleton, which are required for the migratory and invasive phenotype of breast cancer. Taken together these effects of GO on cancer cell metastasis may allow the development of a new approach to treat metastatic breast cancer. The strong optical absorbance of the material in the NIR window prompted many scientists to test this property in PTT against breast cancer [28, 36, 81, 96, 98, 100, 102, 104].

Yang et al. [28] reported the first experiments on this area using a GO-PEG for in vivo PTT and imaging. The imaging modality revealed high uptake of graphene in several xenograft tumor mouse breast cancer models; a robust optical absorbance, an ultra-efficient tumor ablation after intravenous administration under low-power NIR laser irradiation was achieved. No significant side effects were detected reporting the first success in using carbon nanomaterials for efficient in vivo PTT by intravenous administration.

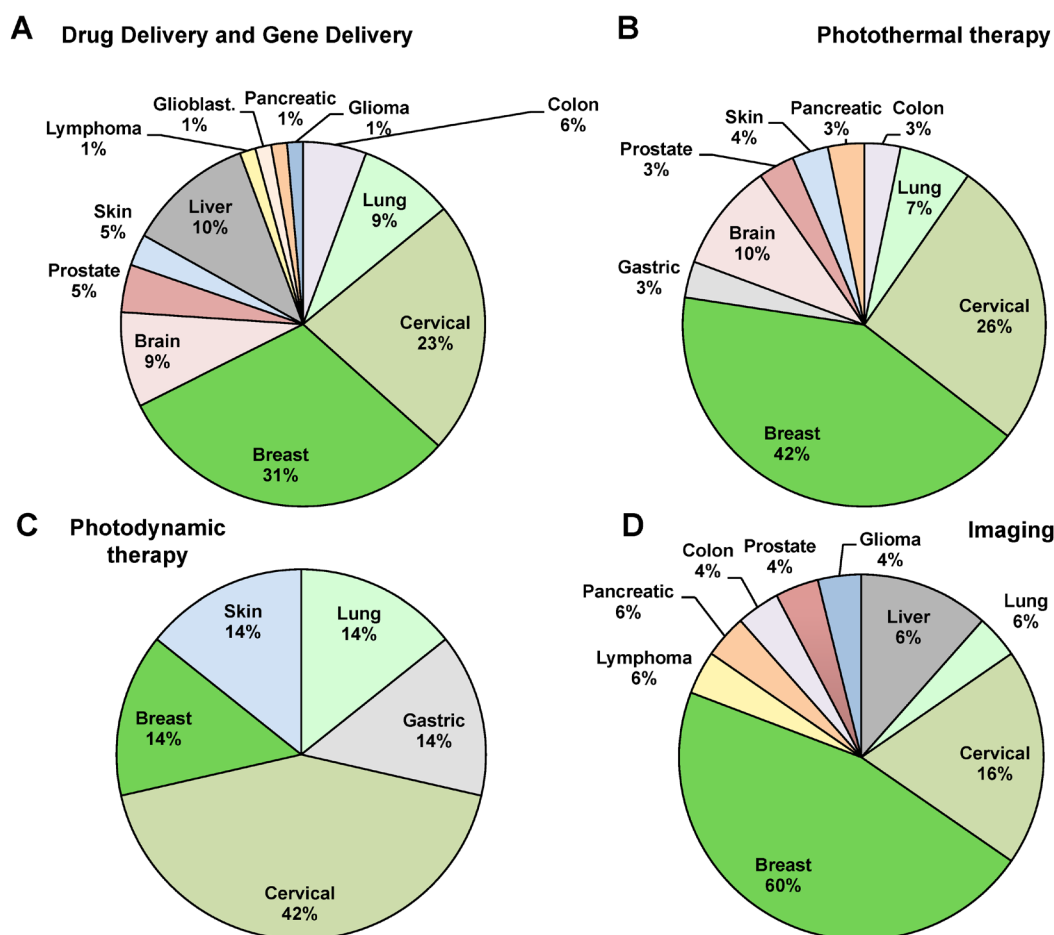


Figure 5. Paper analysis in terms of percentage of manuscripts divided by type of applications and type of cancer.

In the context of cervical cancer, Bai et al. [82] demonstrated that the combination of PTT and drug delivery, can be a potential treatment in the battles against cancer. The authors developed a synergistic therapy based on CuS nanoparticles decorated with a PEGylated GO. GO-PEG/CuS had high storage capacity for DOX and a high photothermal conversion efficiency achieving the ablation of cervical tumor *in vitro* and *in vivo*.

Moreover, Zhang et al. [36] described the use of GO-PEG-DOX conjugate to improve the ablation of tumor both *in vitro* and *in vivo*. Indeed, the ability of the GO-PEG-DOX complex to combine the local specific chemotherapy with external NIR PTT significantly improved the therapeutic efficacy of the cancer treatment. Compared with chemotherapy or PTT alone, the combined treatment demonstrated that the synergistic effect result in a higher therapeutic efficacy. Furthermore, as shown in the Venn diagram different works successfully used graphene for the combination of PTT and imaging modalities (**Figure 2B**) [28, 84, 97, 100, 101, 107].

Photodynamic Therapy

Graphene in the very last few years has been also tested as agent in photodynamic therapy thanks to its physical properties [60, 80, 103, 106, 119]. However, despite PDT is an FDA approved modality for the local treatment of a wide variety of tumor diseases, such as esophageal cancer and lung cancer [120], the number of works that used graphene in PDT are still very few, only 10% of the total (**Figure 2A**) [37, 60, 80, 103, 106, 110, 119, 121].

Otherwise, graphene was used in concert with PDT and imaging in 2 studies (**Figure 2B**) carried out by Sahu et al. [103] and Gollavelli et al. [106] giving new input for future studies on the use of the material for targeting and killing cancer cells also with PDT.

Interestingly, differently from the other applications, 42% of the works in PDT focused on cervical cancer (**Figure 5c**). The other types of cancer explored were breast cancer and gastric cancer. PDT is based on photosensitizers sensitive to light upon suitable irradiation that produces reactive oxygen species (ROS), such as singlet oxygen, free radicals, or peroxides, inducing cytotoxicity. Compared with chemotherapy or radiotherapy, PDT shows relatively minimal side effects and improves tumor specific killing [122].

Despite the few studies in this field, all cited works described the efficient capacity of the carbon material to be loaded with different types of photosensitizers with a high action on cancer cell thanks to the PDT approach [80, 103, 123]. Zhou et al. [80] for example have combined GO with hypocrellin A proposing it as a new second-generation photosensitizer.

However, the loading of GO with hypocrellin A improved the hydrosolubility but reduced the anticancer activity. To solve this problem, GO was co-loaded with a second anticancer agent to perform at the same time two anticancer treatments. Their results showed that the combination of two therapies exhibited a synergistic antiproliferative effect compared with PDT and chemotherapy alone. The majority of the analyzed works combined PDT with other types of anticancer strategies [103, 106]. In the work of Sahu et al. [103] GO was non-covalently functionalized with pluronic block copolymer and complexed with methylene blue, a hydrophilic and positively charged photosensitizer to combine PDT and PTT versus cancer. The release of the photosensitizer from GO surface was pH-dependent and an acidic condition increased the release rate considerably. This nanocomplex showed enhanced uptake by cancer cells than normal cells and, when cells were irradiated with selective NIR laser lights, it induced significant cell death. This work showed the potential of GO for a synergistic combination of PDT with PTT. On the other hand, Huang et al. [119] described the GO absorption of the photosensitizer named Chlorin e6 (Ce6). GO-Ce6 accumulation in tumor cells led to a remarkable photodynamic efficacy on cancer gastric cells upon irradiation. Overall, the works we described showed the great potential of graphene in PDT alone or in concert with other cancer treatments.

Imaging

In the context of imaging, graphene have been explored to improve the diagnosis and also the treatment of cancer with the aim to avoid several side effect related with the current use of toxic chemicals as contrast agents. Most fluorescent molecular dyes (i.e. Qdots), because of their intrinsic toxicity, are not suitable for the diagnosis in many cancer patients that may already have chemotherapy-related damages to liver or kidney [124]. The excellent photostability of graphene-based nanomaterials makes them suitable for many biological imaging techniques such as photoacoustic imaging (PI), ultrasonography (US), magnetic resonance imaging (MRI), computed tomography (CT) and optical imaging applications (see **table 1**) [28, 33, 35, 45, 58, 59, 77, 78, 85, 87, 88, 96, 97, 100-102, 111, 125, 126]. The optical imaging potential of graphene was well studied by many reports [88, 125]. Gao *et al.* reported a GO-based fluorescent magnetic hybrid for loading and delivery of Doxorubicin. They applied GO for *in vitro* tumor cellular imaging and showed high uptake of GO into hepatocellular carcinoma cell line with a strong fluorescence. These data have evidenced the GO abilities as an optical imaging tool [88]. Regarding ultrasonography,

our group showed in a previous study that GO has good echogenic properties with a promising future in the scenario of ultrasound contrast agents [29]. Moreover graphene could be useful also in MRI. The magnetic graphene complexes that could be useful for MRI and, at the same time, for other imaging or therapy modalities are of particular interest [87, 90, 97, 106, 117, 127]. Wang *et al.* used magnetic a graphene complex for metastatic pancreatic cell diagnosis in the lymphonodes. At the same time they were able to directly guide the PTT therapy against cancer cells [117]. Gollavelli *et al.* used the ability of magnetic graphene as a potential theranostic nanocarrier for MRI and fluorescence dual modality imaging and for PDT and PTT [106]. Furthermore, it is interesting to evidence the potential of graphene as an *in vitro* detection tool (IVD); Zhang *et al.* developed a new colorimetric assay for the direct detection of cancer cells using graphene as a signal transducer [128]. Moreover in the IVD field, graphene was also used as a biosensor for molecular marker analysis in cancer diagnosis [129].

We found 31% of the total studies used graphene for cancer imaging and this percentage is expected to grow fast in few years (**Figure 2A**). Furthermore, graphene was almost always used in imaging with the therapy approaches cited before (**Figure 2B**). Thus, many authors, thanks to the good imaging characteristics of graphene, combined with encouraging results diagnosis with therapy (i.e. drug delivery or photothermal therapy) [58, 59, 96, 106, 111, 117, 126]. Moreover, the majority of the works addressed breast cancer as target model cancer with a 60% of the total studies (**Figure 5D**). In accordance with the other applications, the second most investigated tumor for imaging was cervical cancer (**Figure 5D**). Shi *et al.*, [58] for example, studied the specific targeting of functionalized rGO conjugates to murine breast cancer in vivo. The authors used rGO loaded with a specific antibody to endoglin (CD105) for active tumor targeting in living subjects using positron emission tomography (PET) imaging. Hu *et al.* [96] used graphene presenting multiple functions into a single system: imaging, drug delivery and photothermal therapy. In this study NIR potential of GO was combined with the good fluorescence of Qdots. To avoid the fluorescence quenching induced by GO, a spacer was inserted between GO and the dye. This nanosystem was able to kill breast cancer cells and also served as optical indicator to monitor the therapeutic progress by fluorescence imaging. Otherwise, the combinations of GO and Qdots need more studies for its biomedical use in therapy to better understand the possible toxicity of this nanoconjugate. Nevertheless, the use of graphene and Qdots is frequently investi-

gated to improve the imaging ability of graphene. Among the works that combined imaging and PTT, Yang *et al.* [28] published one of the first studies that used this strategy. They found in vivo fluorescence imaging revealed surprisingly high tumor uptake of graphene in several xenograft tumor mouse models. Moreover, to combine the two applications, authors showed a strong optical absorbance in the near-infrared region for in vivo PTT with a good ablation of cancer cells.

The majority of research groups joint the imaging properties with the drug delivery applications. Chen *et al.* used as well as Hu SH *et al.* graphene conjugated with Qdots for targeted cancer fluorescent imaging, tracking and monitoring the delivery of drug into the cancer site [59, 96].

Cornelissen *et al.* [126] used GO coupled with anti-Her2 antibody, amonoclonal antibody for the treatment of breast cancer, and radiolabeled it with [^{111}In]-benzyl-diethylene-triaminepenta acetic acid via π - π -stacking for targeted and functional imaging. This construct [103]has shown an improvement in the targeting and therapy of breast cancer cells in mouse model compared to anti-Her2 alone. Indeed, the authors showed a clear in vivo visualization of the tumor using single-photon emission computed tomography.

Combined therapy and theranostics

All great potential of graphene in many cancer therapies (drug delivery, PTT and PDT) encouraged many authors to test graphene also combining different approaches for cancer treatment (see **table 1**) [36, 37, 60-64, 80-84, 89, 92, 102, 106].

Among this type of works, the higher number of studies (11 in total), herein analyzed, used graphene for combining drug delivery and PTT. Thanks to graphene properties, the authors combined both therapies in a single system enhancing the efficacy of the single modality. A clear example of these enhancements is well explained in the work of Feng *et al.* were the authors used GO as a carrier for siRNA and pDNA [89]. They showed that PTT induced local heating and accelerated intracellular trafficking of GO vectors, opening interesting new applications for combined therapies [89]. The same group, in another work, combined the DOX function with the PTT treatment. They showed a remarkably improved cell killing for drug-resistant cancer cells in comparison with free DOX. Other therapies, such as PTT and PDT, were combined only in three works herein reported. Previously, we described the works of Sahu *et al.* and Gollavelli *et al.* [103, 106]; their findings underlined the potential of GO for a synergistic combination of PDT with PTT.

Drug delivery and PDT were combined in three works [37, 60, 80]. Zhou *et al.* [80] have combined GO with hypocrellin A and Camptothecin. Their results showed that the combination of drug delivery and PDT exhibited a synergistic antiproliferative effect compared with PDT and drug delivery alone. All works taken in consideration on combined therapies displayed very interesting possibilities to reach the ultimate purpose to fight cancer.

The great and innovative property of graphene is its good imaging characteristics that has prompted many Authors to combined imaging with one-therapy applications (i.e. drug delivery or photothermal therapy as shown in figure 2B) [28, 33, 35, 45, 58, 59, 77-79, 85, 87, 88, 90, 96, 97, 100, 101, 107, 117, 121]. The largest number of studies herein cited and commented (12) combined the imaging properties of graphene with the good loading ability in drug delivery and gene delivery (see table 1). The first study that used this approach was Sun *et al.* they explored for the first time the ability of graphene as a theranostics tool indeed they found that the novel graphitic nanostructures, combined with multi-functionalities including biocompatibility, photoluminescence and drug loading and delivery, suggest promising applications of graphene materials in biological and medical areas [33]. This previous report open the way for all the further study that improve this findings improving the ability of graphene also as a combined materials. Thanks to graphene also PTT and PDT were combined with imaging, we found 8 works herein analyzed that used this strategy. Yang *et al.* was the first study that used graphene for PTT and imaging for give the successful ablation of breast cancer [28]. All these studies reached the purpose to fight cancer more effectively showing graphene as one of the most promising nanomaterial to reach the goal of cure cancer. On the other hand, three additional works [84, 102, 106] used graphene for combined therapies and imaging. This type of approach is expected to grow fast in the next years, suggesting an exponentially growing success of graphene for more theranostic applications.

Conclusion and perspectives

In summary, all studies herein analyzed underline the potential of graphene in the theranostic field that allow concomitantly the diagnosis and the therapy of a tumor area.

Many works pointed out that different biomolecules such as siRNA, DNA and anticancer drugs such as doxorubicin can be loaded onto the surface of graphene for gene transfection and drug delivery. Moreover, the property of graphene to adsorb light in the NIR region has been tested from many research

groups in phototherapy for in vivo and in vitro cancer treatment. This action combined with drug delivery and imaging could be used in a synergic treatment of cancer, increasing the targeted killing with less impairment of healthy cells. Furthermore, many studies, thanks to the graphene optical properties and the loading ability, tested this nanomaterial also in the photodynamic treatment. We showed that graphene alone or conjugated with various inorganic nanoparticles such as Qdots, gold nanoparticles, magnetic iron nanoparticles and also loaded with fluorescent dye have all the potential to be used in many types of imaging such as optical imaging, ultrasonography, nuclear imaging and MRI. The great potential of graphene is the ability to provide at the same time many different cancer therapies joined to imaging make graphene one of the most promising next generation theranostic agents. Otherwise, further studies are still needed for the clinical translation of graphene in the context of cancer. In particular, it would be necessary to perform further investigations to prove the absence of toxicity and other side effects for healthy cells. For example, we previously noted the lack of studies focused on carbon materials interaction with immune cells [7, 130-132].

Furthermore, there are many discrepancies between the scientists in the context of toxicity of graphene. Several works reported a good biocompatibility and no cellular damage after exposure to graphene. However, other authors have evidenced cell toxicity with the enhancing of apoptosis and necrosis [133, 134]. These variances in the scientific findings are especially due to the differences in size dimensions, functionalization and purification of the employed graphene samples.

Another point of interest is also related to graphene elimination from the living systems. Indeed how graphene is degraded and excreted is still not very well explained.

A better understanding of graphene and its derivatives behaviors in biological systems is needed to improve its performances also for theranostic applications. Although not only toxicology should be better addressed, but also the functionalization modalities and the conjugations of graphene that are important for its biocompatibility and pharmacokinetic profiles.

Here we give many interesting perspectives for new graphene-based studies about the treatment and diagnosis of various types of cancers, and especially breast cancer.

We believe that graphene is one of the most promising materials destined to change our day life and the future treatment and diagnosis of cancer.

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Abbreviations

CPT: Camptotecin; CT: computed tomography; DOX: Doxorubicin; GO: graphene oxide; MRI: magnetic resonance imaging; NIR: Near infrared region; OXA: oxaliplatin; PDT: Photodynamic therapy; PEG: Polyethylene glycol; PET: Positron emission tomography; PI: Photoacoustic imaging; PTT: Photothermal therapy; PVA: Poly(vinyl alcohol); QDots: Quantum dots; rGO: Reduced graphene oxide; US: Ultrasonography.

Competing Interests

The authors have declared that no competing interest exists.

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